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MONASCUS PURPUREUS IN SILAGE

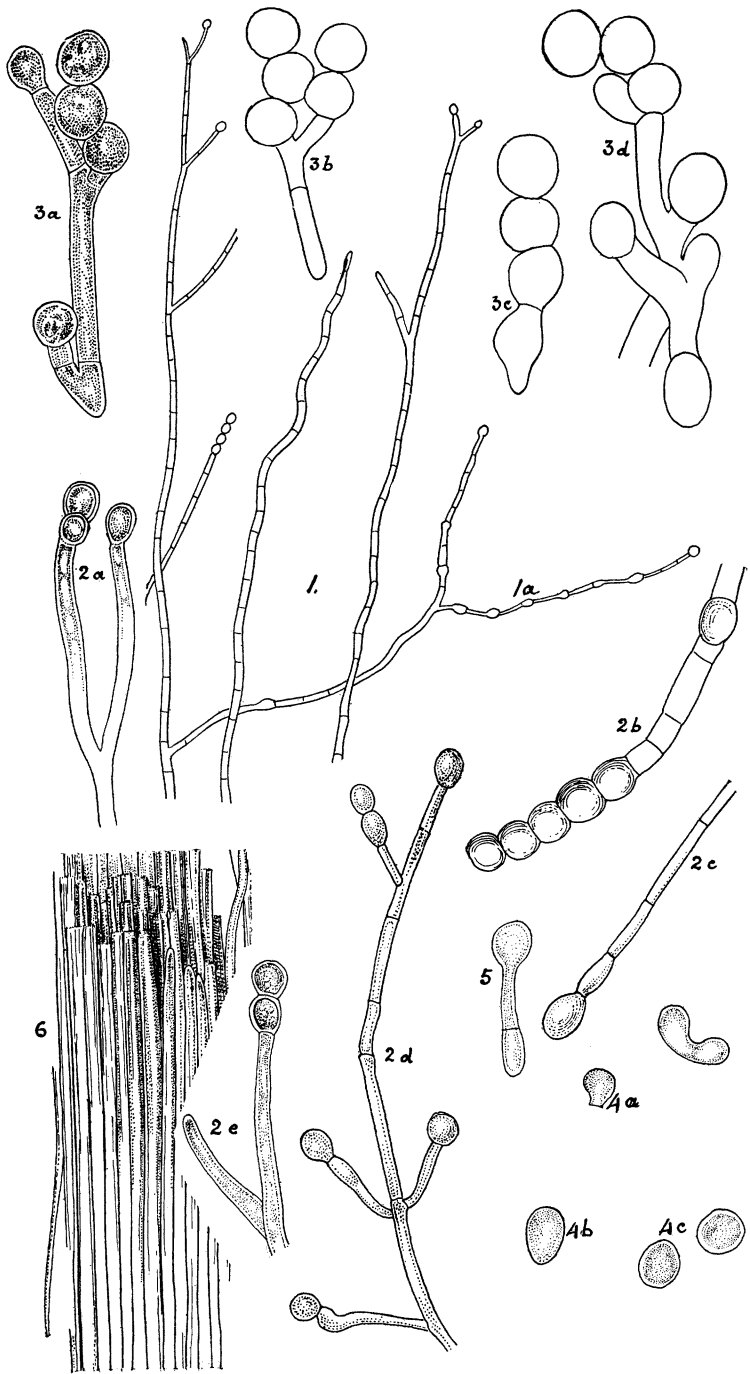
R. E. BUCHANAN

(WITH PLATES 22 AND 23, CONTAINING 31 FIGURES)

During the year 1909, the writer had frequent occasion to examine the molds which are common in silage not properly prepared or cared for. Such moldy silage has in several instances caused the death of farm animals, particularly horses; the symptoms of the disease being those of "forage poisoning" or "equine cerebro-spinal meningitis." Numerous cultures have been made and many molds isolated from different silages, among them several species of *Penicillium*, *Aspergillus*, *Mucor*, the mycelium of a hymenomycete (probably a *Coprinus*), and, in one instance, *Monascus*. The last mentioned was practically the only mold found in one sample. Inasmuch as no record has been found of its occurrence in America and no record of its occurrence in silage, a brief account of the fungus is here given with notes on its morphology and cultural characters.

In March, 1909, a moldy sample of silage was brought to the laboratory by a veterinarian. It was part of the contents of a silo and had been the apparent cause of the death of nine horses that had been fed upon it. Experimental evidence was brought forward later by Dean Stange, of the Department of Veterinary Medicine at the Iowa State College, which demonstrated the causal relationship of this silage to the disease. An examination of the material showed it to be thoroughly infected and matted with the mycelium of *Monascus*. Although no experimental evi-

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dence of any direct relationship between this particular organism and the death of the horses has been shown, the fungus has been thought worthy of study and record on its own account.

GROSS CHARACTERS OF THE MOLD

It is generally known that silage insufficiently packed or too dry when cut is much more apt to mold than that which is moist and well compacted. The material brought to the laboratory was much drier than usual, and matted together by mold into large masses which offered considerable resistance to being torn apart. Examination showed all parts of the silage, leaves, stalks, and ears, to be covered with a white layer of mold, forming cottony masses in some of the spaces. Where it occurred on the kernels of corn, particularly where they had been broken or crushed and the starchy endosperm exposed, the mold often assumed a pink to carmine-red color.

ISOLATION AND CULTURAL CHARACTERS

Silage agar. Five hundred grams of fresh silage was boiled for thirty minutes in one liter of tap water. This was then filtered and the silage on the filter washed with hot tap water until a liter of the decoction was secured. This was autoclaved with one and one half per cent. agar agar threads, filtered, tubed, and sterilized. Dilutions were prepared from the silage mold at points where conidia were found most abundant. These conidia germinated within twenty-four hours in most instances. The mold colonies in the lower dilutions did not develop very far on account of the luxuriance of bacterial growth. In the other plates, however, the bacterial colonies were scattered so that they did not interfere with the normal development of the mold. The inhibition of mold growth in the presence of large numbers of bacteria is a possible explanation of the fact that moist silage decays without becoming moldy through the activity of bacteria, while silage somewhat drier becomes covered with molds. Within the course of a week these mold colonies were from one half to one and a half centimeters in diameter. The outlines of the colonies are very indefinite, for the organism grows almost entirely within the substratum, forming there conidia and peri-

thecia near the center of the colony. However, aërial hyphae are usually produced, forming a cottony surface growth not more than a millimeter in height. Within a few days, the colony, particularly near the center, becomes tinged with red and in two

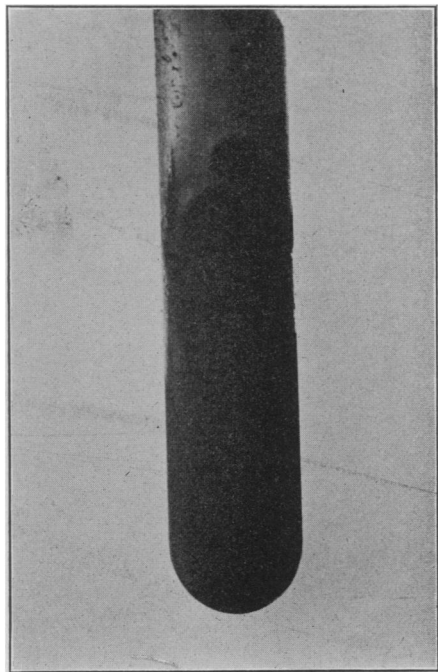


FIG. 1. Colonies of *Monascus purpureus* two weeks old in silage agar.

or three weeks is a deep carmine. This coloring gradually extends throughout the colony, always being deepest near the center.

Silage broth. A decoction of silage was prepared as outlined in the preceding paragraph, and used without further additions in 50 c.c. lots. The organism grows rather slowly in this medium, forming spherical cottony masses of hyphae not easily broken up by shaking. In one to two weeks the hyphae reached the surface of the medium (a distance of about 2 cm.) and two weeks later the surface growth attained a diameter of from 1-4 cm. This surface growth develops large numbers of aërial hyphae, not extending more than one millimeter above the surface. There

is a red pigment produced in some cases, but most cultures remain perfectly white (Fig. 2).

Glycerin solutions. Harz (1890) described *Physomyces heterosporus* from the surface of glycerin vats in a soap factory. Here, as well as in the laboratory, it grew on solutions containing as much as 30 per cent. of glycerin. To determine the ability of

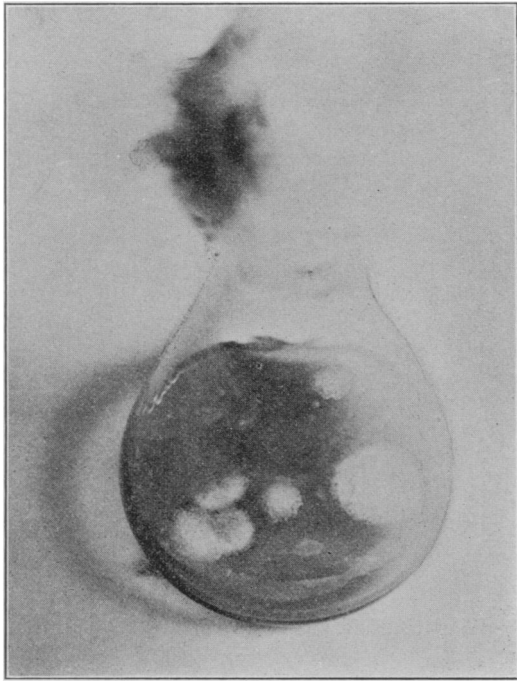


FIG. 2. Colonies of *Monascus purpureus* one month old in silage decoction.

the organism in question to use glycerin, flasks containing 100 c.c. of 5, 10, 20 and 40 per cent. glycerin in tap water were inoculated with pure cultures of *Monascus*. Growth occurred in the 5 and 10 per cent. solutions, but little or none in the 20 and 40 per cent. solutions. In the 5 per cent. solution small masses of mycelium could be observed within a few days, floating in the liquid. These continued to enlarge slowly for two months, at the end of which time they formed a semi-transparent mass of a quarter of the volume of the medium. The interior of such masses was

found to be densely matted, and of a deep carmine color. In a 10 per cent. solution of glycerin growth was slower, the colonies or mycelial balls remaining smaller and more compacted.

Rice flour. A thick paste of rice flour in tap water was prepared and sterilized in test tubes. Growth on this medium was more luxuriant than on any other tried. Within two days after inoculation, mold patches could be observed, as delicate white colonies arising from the surface of a carmine-red medium. The mycelium completely covered the surface within a few days and the medium changed to an orange-red. The surface of a culture a month old is somewhat wrinkled, the fungus forming a gray felt, with the medium itself entirely red.

MORPHOLOGY

The hyphae of the organism vary from 2 to 5 μ in diameter, branching abundantly and rather irregularly. When within the medium or just at its surface, branching is much more abundant than in the aërial hyphae. Under certain conditions, as between broken corn kernels, the hyphae may lie tightly packed side by side (*pl. 22, f. 6*) with little or no branching evident. The mycelium does not produce differentiated vegetative hyphae and conidiophores. The conidia may appear terminal on almost any branch. The hyphae are septate, the cell contents usually granular, and the older cells are vacuolate and contain oil drops.

Barker (1903) has noted the frequent occurrence of swellings on the hyphae of *Monascus*, particularly when the concentration of the solution had increased by evaporation, as in an old hanging drop. That this is not the only cause of such swellings is evident from *pl. 22, f. 1*, which shows their presence on aërial hyphae. They were also found abundantly in the 5 and 10 per cent. glycerin cultures. In size and shape these swellings approach the conidia. On starchy media, and in some others, the red coloring matter is to be found irregularly distributed through the older threads.

Conidia. The conidia are borne singly or produced in basipetal chains of 2-6 or more. They may be found on aërial hyphae, or imbedded in agar or immersed in a nutrient solution. They may even be abstricted by the tips of the filaments which invest the

perithecium. No evidence of the formation of micro- and macro-conidia could be discovered, although considerable variation in size was noted, from 6 to 10 by 7 to 15 μ . The conidia are sometimes tinged with red in old cultures; usually, however, they are colorless (*pl. 22, f. 2, 3*). Germination occurs under suitable conditions within a few hours (*pl. 22, f. 5*).

Ascocarp or "Perithecium." The fruiting body of *Monascus* (or *Physomyces*) was first described by Harz (1890) as a sporangium or sporocarp. More recent writers, as Barker (1903) and Olive (1905), have shown it to be of an ascomycetous type, although this claim has been denied by Ikeno (1903). All are agreed, however, that it is produced as the result of a true sexual fusion. Observations of the form in question seem to indicate that the interpretation given by Olive (1905) is the correct one. Serial sections have not been prepared, however, and the exact sequence of events cannot be accurately determined without a careful study of the subject. The perithecia develop in great numbers upon the hyphae and are generally terminal, though sometimes apparently lateral (*pl. 23, f. 8*). The young perithecia may be found in suitable media within two or three days after sowing the conidia. They develop not only on aerial hyphae, but also in the body of a medium such as agar, and nowhere were they found more abundant than in 5 and 10 per cent. glycerin solutions. So marked is this ability to produce perithecia and conidia under water, that the organism might well be classed as one of the aquatic molds. An antheridial cell fuses with a functioning egg cell, and, within this, are developed the ascogenous hyphae which ultimately form one to many asci, each typically with eight ascospores. The steps in this process can be seen only with difficulty, for the "central cell" soon becomes closely invested by sterile hyphae which branch and apparently anastomose about it.

Various steps in the development of these hyphae may be seen in the figures (*pl. 23, f. 4-10*). Sometimes branches may extend out from this investing mass (*pl. 23, f. 10*) and even produce conidia. These hyphae soon lose their contents and collapse, forming a thin membrane at maturity, which sometimes shows little evidence of its origin. Pl. 23, f. 11 shows a perithecium

nearly mature, with the ascospores grouped within the asci. The walls of the latter soon disintegrate and the ripe perithecium filled with loose spores resembles the sporangium of a phycomycete. These perithecia vary from 25 to 50 μ in diameter. They are usually terminal at the end of a long hypha, but in some media there may be noted variations in the length of this pedicel. The spores within the perithecium number from 6 or 8 to several hundred. They are nearly spherical in shape and from 3.5 to 6 μ in diameter. They are usually tinged with brown or are slightly fuscous when mature.

SPECIFIC POSITION OF SILAGE MONASCUS

There have been described in literature five species of *Monascus*. *M. heterosporus* Schröter (*Physomyces heterosporus* Harz) differs from the form in question in having two types of conidia, having smaller conidia, and developing in much more concentrated solutions of glycerin. *M. ruber* van Tieghem differs in having larger conidia and spores and a red perithecium. *M. mucoroides* van Tieghem has larger perithecia, spores and conidia. *M. purpureus* Went agrees in all essential characters, and this organism is placed here tentatively. *M. purpureus* is the characteristic mold used by the peoples of eastern Asia in the preparation of "red rice" (Ang-quac). The fact that rice covered with this mold is used by the Chinese as food rather militates against the possibility of the form in silage being poisonous. This has not, as before stated, been investigated as yet, and further study may cause a change of view as to its specific position.

SUMMARY

A mold answering to the description of *M. purpureus* Went was found to be the typical fungus present in a moldy silage which killed eleven horses. The pathogenic properties of the organism have not as yet been wholly determined. This appears to be the first record of the occurrence of *Monascus* in this country.

CITATIONS

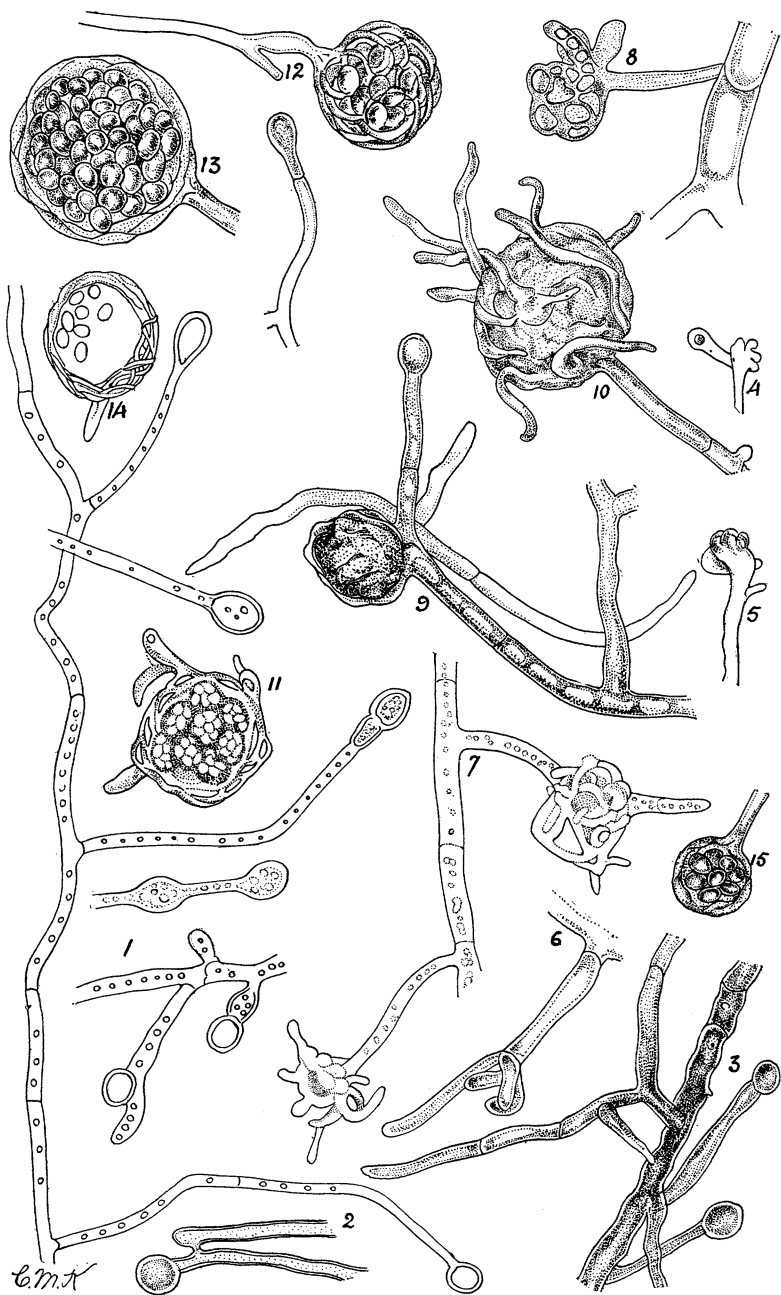
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EXPLANATION OF PLATE 22 (frontispiece)

1. Aërial hyphae with conidia and with swellings at *1a* on a branch.
- 2a, 2b, 2c.* Conidiophores with conidia.
- 3a, 3b, 3c, 3d.* Conidiophores with conidia.
- 4a, 4b, 4c.* Conidia.
5. Conidium germinating.
6. Mass of parallel hyphae from surface of moldy corn kernel.

EXPLANATION OF PLATE 23

- 1, 2. Hyphae with oil drops and conidia from glycerin solution.
3. Hyphae and conidia from silage decoction.
- 4, 5, 6. Very early stages in formation of perithecium.
- 7, 8, 9. Sterile hyphae branching and anastomosing about "central cell."
10. Sterile hyphal covering of perithecium sending out branches. These are sometimes tipped with conidia.
11. Optical section of nearly mature perithecium. Spores still within asci.
- 12, 13, 14, 15. Optical sections of mature and nearly mature perithecia, showing variations in size.



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